EFFECT OF CERTAIN SOIL FACTORS ON NODULATION IN COWPEA (*Vigna sinensis*) AND LENTIL (*Lens esculenta*)

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C E R T I F I C A T E

This is to certify that the dissertation entitled "Effect of Certain soil factors on nodulation in cowpea and lentil" submitted in partial fulfilment of the degree of M.Phil (Ag.) in Microbiology of Agricultural Centre, A.M.U.,Aligarh is a bonafide record of the research work carried out by Ms. Rana Athar under my guidance and supervision. No part of the dissertation has been submitted for any other degree or diploma.

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I N T R O D U C T I O N

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Pulses constitute an important constituent of diets of millions of people in India mostly, the vegetarians. The crops yielding pulses belong to family *Leguminoseae*. They are an important source of protein, (averaging 22-28%, and 45% in soybean) carbohydrates, fats, vitamins and some minerals (Anonymous 1977). The total annual area under pulse crops in India is about 22-23 million hectares and the production is about 11-12 million tonnes of grain (Anonymous 1991).

The protein contents of major pulses are as follows -

Pulses (Common name)	Botanical Name	Protein (%)
Gram	*Cicer arietinum* L.	17.1
Pigeon pea	*Cajanus cajan* L.	22.3
Moong	*Phaseolus aureus* L.	24.0
Pea	*Pisum sativum* Roxb	19.7
Cowpea	*Vigna sinensis* L.	24.6
Horsegram	*Dolichos biflorus* L.	22.0
Soybean	*Glycine max* L.	45.0

Pulses besides, source of proteins to vegetarians are used as a fodder and a green manure crop. Horsegram (*Dolichos biflorus*) and Pillipesare are popular fodder legumes. Moong and cowpea are excellent green manure crops.

Fields in which legumes are grown maintain high population of rhizobia for several years after harvest of the legumes. (Kucey and Hynes, 1989). Leguminous crops are very commonly grown for crop rotation since they are excellent nitrogenous manure and bring about an increase in the nitrogen content of the soil with the help of the nitrogen fixing bacteria present in their root nodules (Verma, 1985). Thus they help in replenishment of soil.

With the help of the symbiotic bacteria of the genus Rhizobium, the crop is able to make use of atmospheric nitrogen, Rhizobium spp. show positive chemotaxis towards legume root exudates and form clouds at the surface of the legume roots (Malek, 1989). Legume root exudates act as biochemically specific chemoattractants for the bacterium eg. luteolin of alfalfa roots act as chemoattractant for Rhizobium meliloti (Dharmtilake and Wolfgang, 1992).

The first step in the Rhizobium-legume nitrogen fixing symbiosis is the infection of the host legume by specific Rhizobium species and curling of the root hairs. Formation of shepherd's crook is a necessary step for the formation of infection thread inside the root hair. Both cellulose fibrils and Ca^{+2} dependent adhesins are involved in the attachment of specific Rhizobium species to host root hair tips. (Smit et al 1987). The curling of the root hairs has been assigned to the production of indole-acetic acid (IAA) in the root region by rhizobia. Aromatic amino acid aminotransferases in Rhizobium leguminosarum play an important role in indole-acetic acid (IAA) synthesis (Perez et al 1989). Rhizobium is host specific.ie.it is capable of producing nodules on a specific group of legumes. This specific recognition is due to plant lectins (proteins) which bind to carbohydrate receptors on the rhizobial cells (Kosenko et al 1989). Rhizobia enter into the root hairs either through the gaps in cellulose microfibrils or by direct invagination of the root hair cells. Entry of rhizobia into roots of Mimosa scabrella occurs between epidermal cells (De Faria et al 1988).

The infection thread in the root hair enters into the cortical cells of the root and branches. The contents of the infection thread is liberated into the tetraploid cells of the root cortex resulting in nodule formation. The disappearance of the infection thread is due to its degradation within large vacuoles which result from fusion of small vacuoles (Bal Arya 1985).A hydrolytic enzyme, which is of rhizobial origin in pea root nodules, is considered to be involved in the sequence of events from infection thread formation to rhizobial release in the host cell cytoplasm (Chalifour et al 1989).

The nodulated legumes contribute a good deal to the amount of nitrogen fixed in the biosphere.eg.clover fix about 130 kg/ha and cowpea 62-128 kg/ha. Not all the legumes bear nodules on their root systems. On the basis of survey of nodulation in plants of 362 genera of the family Leguminoseae, the nodule forming habit is general in the subfamilies Mimosoideae and Papilionoideae and only a small minority of genera and species of the subfamily Caesalpinoideae form nodulation (Corby 1989).

All this process of nodulation occurs in nature in soil, therefore, soil factors influence the development of nodules. The soil factors affecting nodulation can be classified into physical and biotic factors.

Physical Factors

Soil Temperature :

Soil temperature is one of the major environmental factors because it controls the microbiological activity in soil. All the processes involved in the growth of plant-rhizobia relationship are governed by temperature. Temperature below 10°C retard root hair infection by Rhizobium and temperature higher than 24°C promote the same. Low root temperature results in low nodulation (Rice and Olsen 1988). Elsheikh and Wood (1989) pointed out that the tolerance of rhizobia to salts present in soil is dependent upon soil temperature. Sharma et al (1989) pointed out that the high temperature in the semi-arid zones of Haryana could be responsible for the low nodulation of pigeon pea because the plasmid carrying the nodulation genes is cured at 40-45°C giving rise to non nodulating mutants.

Soil Moisture :

Soil moisture is one of the basic requirements of life. It plays an important role in controlling the nodulation in plants. Osa Afiana *et al* (1982) pointed out that the percentage of cells of cowpea rhizobia and *Rhizobium japonicum* surviving was directly related to the quantity of water left in soil following dessication. This was also influenced by relative humidity. Breitenbeck and Yang (1988) suggested that non-saturated flow of soil moisture contributed to the dispersal of rhizobial inoculum in soil . Postma and Vanveen (1989) later pointed out that the initial soil moisture has a direct influence on the distribution and population dynamics of introduced rhizobia. Rhizobial cells survived better in soils with a low moisture, than in soils with a higher initial moisture content. After the onset of rain there was an increase in the no. of resident rhizobia in the soil but after the drying of soil, the no. of rhizobia decreased (Dudeja *et al*. 1989).

Hydrogen Ion Concentration of Soil :

The number and size of nodules in the leguminous plant root is affected by the substrates on which the legumes grow. Leguminous plants grow less luxuriantly in acid medium than in neutral or alkaline medium. Rhizobium species of winged bean perform best at soil pH 5.5. The performance was low when soil pH was either extremely low as 4.5 or high as 7.5 (Iruthayas and Vlassak, 1986). Acid sensitive strains of Rhizobium leguminosarum form a higher proportion of nodules than those classified as acid tolerant (Gemell and Roughly 1993).

Mineral Nutrition :

Potassium ions with low oxygen tension is essential for the expression of several bacteroid related functions in Rhizobium. They have a major controlling influence in bacteroid development (Gober and Kashket, 1984). Potassium is reported to enhance the amount of nitrogen fixed by 85%. The potassium deficiency decreased nodule weight per unit root weight only at later stages of growth of plants (Cadisch et al 1992). Application of additional phosphates in soil resulted in greater nodulation and this increased the fixation of nitrogen in plants (Sanoria and Maurya 1987). Cadisch et al. (1992) reported that phosphorus has a direct effect on nitrogen fixation. They further pointed out that severe phosphorus deficiency leads to a strong reduction in nodule weight. Cobalt is an important constituent for the formation of vit B_{12} which was found predominantly in bacteroids (Troitskaya and Severova 1987). Calcium is known to stimulate the nodulation in plants when it is present as chloride or sulphate. The adverse effect of erosion of soil leading to the loss of calcium affect the survival of Rhizobium in the soil. This, however, can be overcome by supplementing the calcium supply (Habte and El-Swaify, 1988).

Soil Texture :

Texture of a soil depends on percent of sand, silt and clay. Cowpea rhizobia survived better in bauxitic silt loam than in clay loam soil (Aarons and Ahmad 1987). Rhizobial cells declined in loamy sand due to the presence of protozoa but when bentonite clay was introduced into the loamy sand soil it conferred protection of introduced rhizobial cells against predation by protozoa (Heynen et al 1988). El-Mokadem et al. (1989) reported that the nitrogen content in chickpea tissues was higher in plants grown in loamy sand soil than those grown in sandy soil inspite of the plant age possibly due to better survival of rhizobia in loamy sand soil. The persistence of rhizobia on legume seeds could be increased after seed pelleting with clay minerals like kaoline and bentonite (Hoeflich and Ruppel, 1990).

Salinity and Alkalinity :

In many arid and semiarid areas of India, crop production is limited because of salinity and alkalinity or both. Alkalinity of the soil is due to the predominance of carbonates and bicarbonates in the soil. Siddique et al (1986) reported that increased salinity reduced the number and weight of nodules in pea. Salt stress was shown to be more severe at alkaline pH (Elsheikh and Wood 1989). Mirza and Tariq (1993) suggested that nodule mass and number of nodules formed by *Trifolium alexandrinum* decreased with increasing salinity levels. The nodule size, however, remained unaffected when the concentration of sodium chloride was upto 0.4%, but at higher concentration the nodulation was reduced. Nair *et al* (1993) pointed out that salt tolerant rhizobia were less efficient in symbiotic nitrogen fixation under non-saline growth conditions of legume host possibly due to depression in their nitrogenase activity during salt stress. Nodule growth was significantly reduced in treatments involving salt acclimated rhizobia.

Soil Amendments :

Soil can be amended with certain fertilizers, pesticides, herbicides, insecticides and organic matter etc. which influence the microbiological processes in the soil. Insecticides namely, Aldrin and Lindane as seed treatments lead to a decrease in the number of survivors on the seeds of Bengal Gram (*Cicer arietinum*) inoculated with chickpea *Rhizobium*. There is also a reduction in nodule number, yield and nitrogen content at all the concentrations of insecticides (Suneja and Dogra, 1985). Rate of nodulation (in terms of nodule number and dry weight) in *Vigna mungo* was slightly inhibited at lower (1.0 um) and severely inhibited at higher concentrations of urea and ammonium sulphate. The rhizobial population size was inhibited with urea but was stimulated with ammonium sulphate. Thus either form of fertilizers may be applied three weeks after germination at very low concentration (Pandey *et al* 1986). Aggarwal *et al* (1986) reported that low concentration of carbamate pesticides like 1-naphthol, dimetilan and trematan have little effect on nodulation and nitrogen fixation by *Pisum sativum* and *Vigna sinensis* in the presence of their respective rhizobia whereas higher concentration adversely affected these processes. Fungicides exhibit a higher toxic effect on *Rhizobium trifolii*. Captan and Captafol inhibited nodulation of *Rhizobium trifolii* on *Trifolium alexandrinum* in field conditions (Ruiz *et al*, 1986). Amitrol had a very slight inhibitory action on *Rhizobium meliloti* strain. But it had a contrary effect on the capacity of nitrogen fixation in the nodules (Torralba *et al*.1987), Torralba (1987) observed that the herbicide 2,4-D had inhibitory effect on growth of rhizobia but had stimulatory effect on nitrogen fixation.

Biotic Factor

Soil Microorganisms :

Since the rhizobial penetration takes place in soil biosphere, soil microflora is bound to affect nodulation. The antagonistic effect of fungi could be due to mycotoxins produced by them. The nodulation in soybean by rhizobia has been known to be reduced by Trichoderma viridae. On the other hand Mucor vesiculosus increased the nodulation and plant growth while Rhizopus nigricans had no effect on nodulation (Angle et al 1981). Polyanskaya et al (1985) pointed out that when actinomycete Streptomyces olivocinereus and chitin were added to the soil, the nodule bacterium survived at the first stage of growth because nodule bacteria could grow well on the products of Chitin hydrolysis produced by actinomycete. After (50 days) Rhizobium leguminosarum died due to the growth of soil antagonistic actinomycetes. Furgal et al (1988) reported that the growth of Rhizobium leguminosarum was inhibited by culture filtrates of saprophytic fungi like Aspergillus spp. and Fusarium spp.

It is evident from the foregoing that soil factors influence the nodulation in legumes - thus the symbiotic relationship between the rhizobia and the host legumes. When seeds pelleted with rhizobia are sown in soils, varied results are obtained at various places. It is likely that the soil texture and soil type might be different at different places. Moreover, various agronomic practices are used for cultivation of crop but very little is known about the effect of such factors.

Lentil and cowpea are pulse crops which are gaining importanc in recent times. Therefore, it is intended to study the effect of sand-soil concentration with different land management practices such as various levels of irrigations, fertilizer quantity, harrowing, weeding etc. on the symbiotic relationship between the rhizobia and the cowpea.

The detailed plan of work is given in the following chapter.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The symbiotic relationship between the leguminous plants and the bacterium was recognised as early as in 1838 when Boussingault pointed out that, the legumes can obtain nitrogen from atmosphere when grown in soil which was not heated. Later Hellriegel and Wilfarth (1888) discovered the functions of the nodule. Beijerinck (1888) isolated and cultured a microorganism from the nodules of legumes. Winogradsky (1891) discovered the autotrophic mode of life among bacteria and established the microbiological transformation of nitrogen and sulphur.

Since the discovery of the relationship between the bacterium and the leguminous plants, considerable work has been done on the process of infection of bacterium to roots, nodule formation and the factors affecting nodulation.

The first stage of the development of host and the bacteria relationship is the adsorption of the bacterial cells on the surface of the roots. This phenomenon is influenced by several factors, including the host factors and the soil factors. The host factors include besides the genetic factor, the exudations coming out of the roots. There has been a high degree of host specificity in the nodulation by bacterium. The bacterium strain/species for a particular host will cause nodulation in a particular plant only.

This fact was, however, first suggested by Rovira (1969). Aguilar et al. (1988) reported that sucrose was found to have the strongest chemotactic response among the carbohydrates and varrillyl alcohol among the phenolics produced by the plants. Malek (1989), reported that Rhizobium species exhibit chemotaxis towards some amino acids, sugars, and other exudates, and extracts from roots of legume plants and suggested that sugars were better chemoattractants than amino acids but legume root substances were the best ones. This was supported by the fact that a number of R. meliloti cells were observed at the surface of alfalfa roots. Dharmtilake and Wolfgang (1992) pointed out that certain chemicals produced in legume root exudates act as biochemically specific chemoattractant for the bacterium, eg. luteolin, a flavone produced by alfalfa plants, serves as a chemoattractant for R. meliloti. Cell surface constituents of the rhizobia and legume roots are important in initiating the process of infection. This was first realised by Hamblin and Kent (1973) who reported that bean lectin combine specifically with R. phaseoli. Chen et al. (1976) found out that the rhizobia which do not nodulate, are not bound by lectins. Bhuvaneshwari et al. (1977) pointed out that there exists a strong correlation between the nodulating and lectin binding capacities of R. japonicum. Kosenko et al. (1989) reported that specific recognition between the rhizobia and root surface is due to plant lectins (proteins) which bind to carbohydrate receptors on the rhizobial cells. Dazzo *et al.* (1985) further pointed out that specificity of *Rhizobium* to host plant roots could be trifolin A in clover plants which helps in the process of recognition. The production of extracellular microfibrils by the host cells firmly anchor the bacterium to the root hair tips. Anolles *et al.* (1986) and Smit *et al.* (1987) confirmed the role of binding sites on the root surface in the process of infection.

As a result of infection by *Rhizobium*, the first response of the host plant appears to be curling or controlled growth and branching of the root hairs (Ward 1887, Nutman 1959). Bohlool *et al.* (1974) further pointed out that there was little or no adhesion between *Rhizobium* and plant of heterologous cross inoculation group. The curling of root hairs has been attributed to be due to the presence of growth promoting substances (Perez *et al.* 1989). McCoy (1932) suggested that bacterium enters into root hairs through mechanical injuries. Fahraeus (1957), however, reported that infection occurs near the contact point of the two adjacent cells and the first sign of infection is usually a hyaline spot or swelling in the root hair wall. Fahraeus and Ljunggren (1968) pointed out that *Rhizobium* strains capable of infecting a legume release a specific polysaccharide which induces pectolytic activity by the root and causes loosening of the root hair wall structure thus facilitating the infection thread initiation. Chandler _et al._ (1982) observed that in _Stylosanthes_ spp. nodule formation occured only at the lateral root junctions which was the result of direct invasion by rhizobia through spaces between epidermal cells. The invasion of the host cortical cells was through altered cell walls. DeFaria _et al._ (1988), however, suggested that the entry of rhizobia into roots of _Mimosa scabrella_ occurs between epidermal cells. Bal Arya (1985) observed that profuse vacuolation takes place in the soybean root nodule cell where infection thread carry rhizobia. The infection thread disappears on the release of rhizobia in the cell. Chalifour _et al._ (1989) pointed out that a hydrolytic enzyme of rhizobial origin in pea root nodules is considered to be involved in the sequence of events from infection thread formation to rhizobial release in the host cell cytoplasm.

Prakash _et al._ (1986), while studying bacteroid branching, found that branched and ellipsoidal bacteroids in _Pisum sativum_ root nodules were induced by _R. leguminosarum_ The entire process of nodulation in roots occurs in soil. Therefore it is bound to be influenced by soil factors. The factors affecting the nodulation have been classified as physical and biotic factors.

Physical Factors

Soil Temperature :

Amongst the different soil factors, soil temperature is one of the important soil factor, affecting nodulation. In many tropical soils, the surface temperature is often 40°C or more resulting in the decline of rhizobia. (Iswaran *et al*. 1970). Lipsanen *et al*. (1986), while studying the adaptation of red clover (*Trifolium pratense*) rhizobia to low temperature, reported that differences existed between the strains in their ability to nodulate the host plant at different temperatures. Rice and Olsen (1988), pointed out that low root temperature resulted in substantially low nodule number than the higher root temperature. Sharma *et al*. (1989) pointed out that the high temperature in the semiarid zones of Haryana could be responsible for the low nodulation of pigeon pea because the plasmid carrying the nodulation genes is cured at 40°-45°C giving rise to non-nodulating mutants. Tolerance of rhizobia to salts present in soil is dependent upon soil temperature (Elsheikh and Wood, 1989).

Soil Moisture :

Soil moisture and water potential are considered solely from the point of view of survival of rhizobia in the soil. Foulds (1971) observed a marked decline of rhizobia on drying the soil but the rate and extent of population reduction varied with the soil and the test bacterium. Osa-Afiana *et al.* (1982) reported that the percentage of cells of cowpea rhizobia and *R. japonicum* surviving was directly related to the quantity of water left in soil, following dessication. Breitenbeck and Yang (1988) suggested that the non-saturated flow of soil water contributed to the dispersal of rhizobial inoculum in soil . Postma and Vanveen (1989) reported that the initial soil moisture has a direct influence on the distribution and population dynamics of introduced rhizobia. Rhizobial cells survive better in soils with a lower, than in soils with a higher initial moisture content. After the onset of rain there was an increase in the number of resident rhizobia in the soil but after the drying of soil the number of rhizobia decreased (Dudeja *et al.* 1989). Yousef *et al.* (1989) while studying the interactive effects of inoculation and irrigation on growth and yield of mung bean (*Vigna radiata* L.) observed that irrigation with 80% of the evapotranspiration (Etp) gave significantly the highest yield of seed and plant top dry matter. Inoculation with the specific *Rhizobium* resulted in higher seed and dry matter yield than the uninoculated plants. Venkateshwarlu and Ahlawat (1993) reported that irrigation at a ratio of 0.60 IW : CPE (irrigation water depth : cumulative pan evaporation ratio) with two irrigations in lentil (*Lens culinaris*) produced taller plants with profuse branching and root nodulation, and had more dry matter, pods/plant and grain and straw yields compared with irrigation at 0.35 IW : CPE ratio and no irrigation.

Hydrogen Ion Concentration of Soil :

Munns *et al.* (1977) pointed out that the use of legumes and rhizobia that are adapted to soil acidity reduces the need for inputs of lime. Mengel *et al.* (1978) reported that the critical pH for most of the soils, was in the range of pH 4.6 to 4.8 and that the nodule number and weight, and the nitrogen content of plant tissues increased significantly as the soil pH increased. Rai *et al.* (1983) observed that the range of soil pH and associated acidity factors in which nodulation and nitrogen fixation responded, varied depending on mutant strains in *Rhizobium leguminosarum* associated with *Lens esculanta*.

Iruthayas and Vlassak (1986) studied the effect of soil pH on nodulation and nitrogen fixation of winged bean (*Psahocarpus tetragonalotus*). They reported that the *Rhizobium* species specified for winged bean perform best at soil pH 5.5 and the performance was low when soil pH was either extremely low as 4.5 or high as 7.5. Helemish *et al.* (1987) found that *R. leguminosarum* TAL 271 could grow in a wide range of pH and tolerated both moderate acidity and alkalinity with optimum pH of 5.5.Caetano *et al.* (1989) observed that the adsorption of *R. meliloti* to alfalfa (*Medicago sativa*) roots require neutral pH. Coll *et al.* (1989) pointed out that the growth of arrow leaf clover was less on soils with reduced pH. Acid sensitive strains of *R. leguminosarum* form a higher proportion of nodules than those classified as acid tolerant (Gemell and Roughly, 1993).

Mineral Nutrition :

Andrew and Nooris (1961) reported that nodule formation was calcium sensitive in both tropical and temperate legumes. The survival of tropical legumes on very poor soil was due to their ability to extract from the soil more calcium than temperate legumes. Growth and activity of *Rhizobium* species as well as effective nodulation requires an adequate supply of calcium (Freire, 1976). Almendras *et al.* (1988) reported that the nodulation of subterranean clover by two of the serogroups 6 and 36 of *R. trifolii* was differentially influenced by an application of calcium carbonate which raised the pH of the soil from 5 to 6.5. Liming the soil with either calcium carbonate, calcium hydroxide, magnesium oxide, significantly increased the percent nodule occupancy. The adverse effect of erosion of soil leading to the loss of calcium affect the survival of *Rhizobium* in the soil. This, however, can be overcome by supplementing, the soil with calcium supply (Habte and El-Swaify, 1988). Phosphorus deficiency is an important limiting factor for nitrogen fixation and legume production. Application of additional phosphates in soil resulted in greater nodulation and this increased the fixation of nitrogen in plants (Sanoria and Maurya, 1987). Idris *et al.* (1988) reported that application of phosphate at 60, 90, 120 kg/ha to the Bragg variety of soybean inoculated with a mixture of 3 strains of *R. japonicum* in silty loam soil significantly improved the number of nodules per plant and decreased production of shoots and roots. Cobalt is an important constituent for the formation of vit. B_{12} which was found predominantly in bacteroids (Troitskaya and Severova, 1987). Potassium ions with low oxygen tension is essential for the expression of several bacteroid related functions in *Rhizobium*. These have a major controlling influence in bacteroid development (Gober and Kashket, 1984). Mowad *et al.* (1987) while studying the response of faba beans to rhizobial inoculation and fertilization with micronutrients in sandy soil reported that rhizobial inoculation and micronutrient fertilization either alone or in combination, improved seed yield, total plant dry weight, nodule weight and nitrogen content of the leguminous plants. They further pointed out that the deficiency of Molybdenum (Mb) or Iron (Fe) showed negative effect on nitrogen fixation and plant growth as compared to Manganese (Mn) or Zinc (Zn) deficiency. Potalia *et al.* (1987) pointed out that the application of Zinc (Zn) and Molybdenum (Mb) depressed nitrogen stress in soil when combined with rhizobial inoculant.

Simon *et al.* (1990) while studying the effect of titanium in the form of titanium ascorbate on *Rhizobium japonicum* reported that 1 um and 2 um titanium ascorbate enhanced the growth rate and dry matter of these strains, but inorganic forms of titanium had no significant effect. Potassium is reported to enhance the amount of nitrogen fixed by 85%. The potassium deficiency decreased nodule weight per unit root weight only at later stages of growth of plants (Cadisch et al. 1992).

Soil Texture :

Texture of a soil is another important soil factor for the growth and survival of bacteria in the soil. Dialtof (1967) reported that during wet periods, in a black earth soil, aeration was the limiting factor for the nodulation of cowpea, soybean and native legumes. Aarons and Ahmad (1987), while studying the growth and survival of cowpea rhizobia in bauxitic silt loam and sandy clay loam soil reported that cowpea rhizobia survive better in bauxitic silt loam than in clay loam soil. Heynen et al. (1988) pointed out that protozoa are responsible for the decline of rhizobial numbers in loamy sand but when bentonite clay was introduced into the loamy sand soil it conferred protection of introduced rhizobial cells against predation by protozoa. El-Mokadem et al. (1989) reported that the nitrogen content in chickpea tissues was higher in plants grown in sandy soil inspite of the plant age possibly due to better survival of rhizobia in loamy sand soil. The persistence of rhizobia on legume seeds could be increased after seed pelleting with clay minerals like

Kaoline and bentonite (Hoeflich and Ruppel, 1990).

Heijnen et al. (1993) found that the presence of bentonite clay increased the growth rate of rhizobia introduced into sterile soil. Further the survival studies performed in non-sterile bentonite amended loamy sand showed that the use of high inoculum densities increased the final survival levels of introduced rhizobia.

Salinity and Alkalinity :

Khaildva et al. (1985) while studying the reaction of Rhizobium meliloti strains to various concentrations of sodium chloride (NaCl) in a nutrient medium found that all the strains of R. meliloti having varying degrees of virulence were characterized by high salt resistance. The strains grown in high salt percentage medium (3%) retained the ability to inoculate the host plant and to fix atmospheric nitrogen. Kassem et al. (1986) reported that the host plant and the symbiotic nitrogen fixation process was more sensitive to sodium chloride (NaCl) than the bacteria themselves. Siddique et al. (1986) observed that increasing the concentration of salt reduced the number and weight of the nodules in pea in sand culture. Desalinization (45 days after sowing) increased the production and growth of the nodules. Salinity also reduced the formation of leghaemoglobin in the nodules and brought about an ehhanced senescence whereas desalinization reversed these effects to varying levels. Salt stress was shown to be more severe at alkaline pH (Elsheikh and Wood, 1989). El-Shinnawi et al. (1989) pointed out that sodium chloride (NaCl) was generally inhibitory towards plants and bacteria. Number and characteristics of plant root nodules, dry weight of plants, nitrogen content of plants and bacterial colony count decreased. Chlorides were most inhibitory to nodulation in soil and carbonates in the culture medium while sulphates were least inhibitory in either case. Formation of effective nodules on roots of plants grown in salinized soils was very poor. Mirza and Tariq (1993) suggested that nodule mass and number of nodules formed by Trifolium alexandrinum decreased with increasing salinity levels. The nodule size remained unaffected when the concentration of sodium chloride (NaCl) was upto 0.4%, but at higher concentration the nodulation was reduced. Nair et al. (1993) pointed out that salt tolerant rhizobia were less efficient in symbiotic nitrogen fixation under non-saline growth conditions of legume host possibly due to depression in their nitrogenase activity during salt stress. Nodule growth was significantly reduced in treatments involving salt acclimated rhizobia.

Soil Amendment :

Kakralia et al. (1981) found that ceresan inhibited growth of R. leguminosarum at 1000 ppm while brassicol did not. Brassicol or ceresan at 2 and 20% had no adverse effect on number but decreased fresh weight and volume of nodules. The yield was however, reduced at 2 and 20% of ceresan. Daramola *et al.* (1981) while studying the effect of three herbicide formulations ie Preforan (2,4, dinitro-4-trifluoromethyl diphenyl ether), Dacthal (dimethyl 2,3,5,6, tetrachlorotetraphalate) and Dual (2-ethyl-6-methyl-N-2-methoxy ethyl chloro-acetanilide) on *Rhizobium* legume symbiosis reported that highest rate of dacthal and pre-foran decreased nodulation. He further pointed out that highest rate of dual completely killed the plants within 14 days of planting. Suneja and Dogra (1985) pointed out that both aldrin and lindane seed treatments lead to a decrease in the number of survivors on the seeds of Bengal gram (*Cicer arietinum*) inoculated with chickpea *Rhizobium*. Nodule number, yield and nitrogen content were reduced at all concentrations of insecticides used in soil in comparison with the control.

Rate of nodulation (in terms of nodule number and dry weight) of *Vigna mungo* was slightly inhibited at lower (1.0 um) and severely inhibited at higher concentrations of urea and ammonium sulphate. The rhizobial population size was inhibited with urea but was stimulated with ammonium sulphate, thus either form of fertilizers may be applied three weeks after germination at very low concentration (Pandey *et al.* 1986). Torralba *et al.* (1986) observed that atrazine reduced the number of nodules on legume roots. Aggarwal *et al.* (1986) reported that low concentration of carbamate pesticides like 1-naphthol, dimetilan and trematan have little effect on nodulation and nitrogen fixation by Pisum sativum and Vigna sinensis in the presence of their respective rhizobia whereas higher concentration adversely affected these processes. Fungicides exhibit a higher toxic effect on R. trifolii. Captan and captafol inhibited nodulation of R. trifolii on Trifolium alexandrinum in field conditions (Ruiz et al. 1986).Amitrol had a very slight inhibitory action on R. meliloti strain. But it had a contrary effect on the capacity of nitrogen fixation in the nodules (Torralba et al., 1987). Torralba et al. (1987) observed that the herbicide 2,4-D had inhibitory effect on growth of rhizobia but had stimulatory effect on nitrogen fixation. Gupta et al. (1988) while studying the effect of bavistin on nodulation and yield of chickpea pointed out that the highest number of nodules per plant and highest grain yield were recorded with treatment of 0.1 per cent bavistin followed by rhizobial inoculation in chickpea. Martensson et al. (1989) reported that the inhibition of nodulation and nitrogen fixation in red clover (Trifolium pratense) grown in the presence of herbicide chlorsulfuran was possibly due to adverse effects of the herbicide on plant growth and development. Treatment with phosphamidon inhibited rhizobial growth temporarily but stimulated nitrogenase activity which declined with passage of time in culture medium (Mathur et al. 1989).

Abd-Alla et al. (1993) while studying the effect of herbicides brominal and gramoxone on the growth and nodulation of *Vicia faba* found that all doses of herbicides significantly reduced the plant growth (dry matter), nodulation, total nitrogen and carbohydrate contents of shoot and roots. Proteins, leghaemoglobin and carbohydrate content of cytosol and bacteroid fraction were significantly inhibited when the plants were treated with doubled doses of these herbicides.

Biotic Factor

Soil Microorganisms :

Mosse (1976) observed that vesicular arbuscular mycorrhizae (VAM) formed a network of fungal hyphae around the infected roots which increase the absorbing capacity of phosphate in different legumes. He further pointed out that growth and nodulation of various species of legumes were stimulated by mycorrhizal infection. There are fungi and bacteria which have favourable while others have antagonistic effect. The antagonistic effect of fungi could be due to mycotoxins produced by them. The nodulation in soybean by rhizobia has been known to be reduced by *Trichoderma viridae*. On the other hand *Mucor vesiculosus* increased the nodulation and plant growth while *Rhizopus nigricans* had no effect on nodulation (Angle *et al.* 1981). Polyanskaya *et al.* (1985) reported that by adding actinomycete, *streptomyces olivocinereus* and chitin to the soil, the nodule bacterium survived at the first stage of growth because the nodule bacteria could grow well on the products of Chitin hydrolysis produced by actinomycete. *R. leguminosarum* was later killed due to the growth of soil antagonistic actinomycete. Vesicular Arbuscular mycorrhizae (VAM) fungus of *Glomus* spp. stimulated nodule development and activity under drought stress in soybean (Gabor *et al.* 1987). Furgal *et al.* (1988) while studying the effect of culture filtrates of the saprophytic fungi on the growth of *Rhizobium leguminosarum* reported that the growth of *R. leguminosarum* was inhibited by culture filtrates of saprophytic fungi like *Aspergillus niger*, *Fusarium solani*, *Penicillium notatum* in pea plants. Li *et al.* (1988) pointed out that the co-inoculation of legumes with antibiotic producing bacteria (*Pseudomonas* spp.) and root nodule bacteria resistant to these antibiotics was a promising means of promoting nodulation and possibly nitrogen fixation. Poi *et al.* (1989) while studying the response of chick pea (*Cicer arietinum*) to combined inoculation with *Rhizobium*, phosphate solubilizing bacteria (*Bacillus polymyxa*) and *Glomus fasciculatum* reported that *G. fasciculatum* and phosphobacterium can greatly assist symbiotic nitrogen fixation as well as phosphate uptake by chickpea particularly when they are grown in soils containing insoluble phosphate.

M A T E R I A L S   A N D   M E T H O D S

MATERIALS AND METHODS

Seed Material and Bacterization

Seeds of cowpea and lèntil cultivars recommended for this area and the rhizobial culture for cowpea & lentil will be obtained from District Agriculture Office, Aligarh. The seeds will be surface sterilized in 1:1000 parts mercuric chloride and rinsed three times with sterilized distilled water. The bacterization of seeds will be done by placing the seeds in a slurry of 25 gm of rhizobial culture and 25 gm of sugar in 100 ml of sterilized distilled water. The seeds will be dried at room temperature before sowing. Throughout the studies seeds so treated will be sown in soil previously autoclaved.

1. Effect of time of sowing on nodulation :

The seeds after bacterization will be sown after every fifteen days interval beginning from 15th Feb. - 15th June. Observations will be made after 45 days of sowing of seeds.

2. Effect of different concentration of fertilizers and mode of application :

Ammonium sulphate will be taken as a source of nitrogen, muriate of potash as source of potash and superphosphate as source of phosphorus. Three concentrations of each of these fertilizers will be used namely as normal recommended by department of agriculture, double the normal, and half of the normal. These fertilizers would be added to the soil to which bacterized seeds would be sown. Doses shall be as follows -

	Nitrogen	Phosphorus	Potassium
1.	N	P	K
	0	0	0
2.	N	P	K
	1/2	0	0
3.	N	P	K
	1	0	0
4.	N	P	K
	2	0	0
5.	N	P	K
	0	1/2	0
6.	N	P	K
	0	1	0
7.	N	P	K
	0	2	0
8.	N	P	K
	0	0	1/2
9.	N	P	K
	0	0	1
10.	N	P	K
	0	0	2

11.	N	P	K
	1/2	1/2	1/2
12.	N	P	K
	1/2	1	1/2
13.	N	P	K
	1/2	2	1/2
14.	N	P	K
	1	1/2	1/2
15.	N	P	K
	2	1/2	1/2
16.	N	P	K
	1/2	1/2	1
17.	N	P	K
	1/2	1/2	2
18.	N	P	K
	1	1	1
19.	N	P	K
	1/2	1	1
20.	N	P	K
	2	1	1
21.	N	P	K
	1	1/2	1
22.	N	P	K
	1	2	1

23.	N	P	K
	1	1	1/2
24.	N	P	K
	1	1	2
25.	N	P	K
	2	2	2
26.	N	P	K
	1/2	2	2
27.	N	P	K
	1	2	2
28.	N	P	K
	2	1/2	2
29.	N	P	K
	2	1	2
30.	N	P	K
	2	2	1/2
31.	N	P	K
	2	2	1

Observations will be made after 45 days of sowing.

3. <u>To study the effect of number of irrigations and amount of water added to the pot</u> :

Studies will be carried out in 30 cm pots. The bacterized seeds will be sown as mentioned above. Seedlings will be irrigated with -

1/2 litre of water one time each day.

1/2 litre of water two times each day.

1 litre of water one time each day.

1 litre of water two times each day.

2 litre of water one time each day.

2 litre of water two times each day.

Observations will be made after 45 days of sowing of seeds.

4. Effect of depth of ploughing on nodulation :

Fields will be divided into plots 2x2 m and it will be ploughed 1" deep, 2" deep and 4" deep by simple hand operated plough. The seeds after bacterization will be sown. Observations will be made after 45 days of sowing.

5. Effect of summer ploughing and fallowing :

To study the effect of summer ploughing and fallowing both in summer and winter on the development of nodules, some of the plots will be ploughed during hot summer and will be left uncultivated. Unploughed soil will serve as a control. Bacterized seeds will be sown. Observations will be made after 45 days of sowing of seeds.

6. In order to study the effect of alkalinity and sodicity on the development of nodules, alkaline and sodic soils will be collected from fields around Aligarh. Seeds after bacterization will be sown. Observations will be made after 45 days of sowing.

Observations

Throughout the studies observations with regard to growth of the plant and nodulation will be made after 45 days of sowing. Plants will be uprooted, length of root and shoot, fresh and dry weight of the plant will be determined. Plants will be washed in running water and placed on blotting paper so that extra water is soaked. Length will be determined in cm.and mm.and fresh weight on the weighing pan. The plants will be dried at 60°C in oven and after having cooled at room temperature, dry weight will be determined. There will be ten replicates for each treatment and each study will be repeated thrice. Nodulation will be determined by counting the number of nodules and size of nodules. Data will be subjected to statistical analysis to determine the significance.

Leghaemoglobin content of nodules will be determined as follows

Benzidine hydrogen peroxide method (Proctor, 1963) will be used for the estimation of leghaemoglobin.

Nodules will be picked up from the roots and washed thoroughly with double distilled water. Fresh nodular tissue weighing 500 mg will be crushed and homogenized in 9 ml of tris-acetic acid buffer |0.1 M acetic acid plus 0.2 M tris (hydroxylmethyl) amino methane to obtain a pH of 4.0|. The homogenate will be kept overnight in a deep freezer to get complete extraction of leghaemoglobin.

The homogenate will be shaken from time to time to facilitate extraction. The volume of homogenate will be made upto 10 ml by adding tris acetic acid buffer and centrifuged at 3000 rpm for 20 minutes. The supernatant will be collected in a test tube. An aliquot of 0.5 ml of supernatant will be diluted to a final volume of 4.0 ml with tris acetic acid buffer. To this, 2 ml of freshly prepared benzidine reagent (100 mg benzidine with 0.5 ml of hydrogen peroxide of 100 percent by volume added to 50 ml absolute alcohol) will be added. The colour density will be measured at 660 nm on spectrophotometer. The results will be expressed in bovine haemoglobin equivalents.

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